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16. Abstracts Chemical compounds foreign to mammalian liver stimulate the hepatic microsomal system to increased, but nonspecific, enzymatic activity. The stimulatory effect on liver enzymes being non-specific, increased hepatic activity results also in an accelerated metabolism of normal body constituents and a more rapid rate of utilization of administered drugs. One substance, nitrilotriacetic acid, an effective chelating agent used as a partial substitute for phosphate in detergents, has not been thoroughly tested for metabolic effects on mammalian microsomal systems. Should undergraded NTA be discharged into the environment in sizable amounts and concentrate in potable water supplies, cumulative exposures of man to the chemical could result in chronic illness or debilitrating physiological states. In an attempt to evaluate the metabolic effects of this foreign substance on mammalian systems, the authors have studied several enzymes in the liver and intestine of mice subjected to chronic oral administration of the sodium salt of nitrilotriacetic acid in drinking water at a concentration ten times the expected environmental level.

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Study of the Effects of Nitrilotriacetic Acid on Mammalian Liver and Intestinal Enzymes

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enhanced following an initial exposure of the mammalian system to chemical stimulators, whether they be drugs or organics of another nature. Most of these substances are characteristically lipid soluble, cyclic or polycyclic in structure, and tend to speed up their own metabolism upon repeated administration (9-11). Increased levels of liver microsomal enzymes are observed in 5 to 15 days following an initial exposure (9,10,12), while induction of cytochromes coupled to the TPNH- and DPNH- cytochrome reductase systems may be detected within hours, reaching a maximum in 24 to 48 hours (9,13,14).

The stimulatory effect on liver enzymes being non-specific, increased hepatic activity results also in an accelerated metabolism of normal body constituents (15-18) and a more rapid rate of utilization of administered drugs (10-12). Thus, uncontrolled or repeated exposure of man to foreign chemicals creates a potential hazard of undetermined magnitude, particularly with respect to those compounds rapidly converted to toxic end-products. There is an unlimited possibility for the occurrence of toxic interactions, considering the wealth of chemicals continually being introduced into the environment. One substance, nitrilotriacetic acid, an effective chelating agent used as a partial substitute for phosphate in detergents, has not been thoroughly tested

In an attempt to evaluate the metabolic effects of this foreign substance on mammalian systems, we have studied several enzymes in the liver and intestine of mice subjected to chronic oral administration of the sodium salt of nitrilotriacetic acid in drinking water at a concentration ten times the expected environmental level. Glucuronyl transferase, nitroreductase and cytochrome P-450 were selected as typical of activities which might be increased during the metabolism of a foreign compound in liver (6,9,13). In addition, we studied two enzymes typical of activity in intestine, that is alkaline phosphatase and trypsin, which might be inhibited if essential cations were chelated by NTA.

EXPERIMENTAL PROCEDURES

Animals and Treatment. Male Swiss, white, random-bred mice weighing 25-30 gm were purchased from Gofmore Farms, Westboro, Massachusetts. They were housed six to a cage, in polystyrene disposable cages, on Absorb-Dri animal bedding purchased from Ward's Natural Science Establishment, Rochester, New York. The mice were fed Purina Lab Chow pellets and were provided water ad libitum. During the 45-day treatment period, control mice were provided distilled water to drink and the experimental group was given distilled water containing 30 ppm trisodium nitrilotriacetate supplied by Hampshire Chemical, Division

Tissues Samples. Mice were weighed and killed by cervical dislocation. The liver was removed rapidly, dropped onto filter paper moistened with ice cold 0.15 M KCl and kept cold on a watch glass set in ice. All glassware and solutions were kept in ice at 0 -4 C. The liver was trimmed, blotted rapidly, transferred to a tared beaker and weighed. One-two ml of 0.15 M KCl was added and the beaker was packed in ice to be processed further as described below.

Immediately after removal of the liver, an 8 cm segment of duodenum, measured from the pylorus, was excised. Contents of the duodenum were rinsed through with saline into a polycarbonate tube and centrifuged for trypsin determination. The intestinal segment was weighed, cut into small pieces and homogenized in about 2 ml saline in a Virtis Micro-homogenizer. The homogenate was transferred to a polycarbonate tube with several saline washes and centrifuged for alkaline phosphatase determination.

Preparation of Liver Microsomes. The liver was transferred to a glass homogenizing tube with rinses totaling 2-5 ml of 0.15 M KCl and homogenized for 3 minutes by means of a teflon pestle fitted into a cone-driven stirring motor. The suspension was transferred to a polycarbonate tube with additional volumes of 0.15 M KCl, resulting in a

Nitroreductase Activity of Liver Microsomes. Enzyme activity was measured essentially according to the procedure of Fouts and Brodie (6) with p-nitrobenzoic acid as the substrate and reduced TPN as a cofactor. The incubation mix contained the following reactants in a final volume of 1.0 ml.

p-nitrobenzoic acid, 1.0 μMoles added in	0.1 ml
Nicotinamide, 20.0 μMoles added in	0.1 ml
TPNH, 0.2 μMoles added in	0.1 ml
Phosphate buffer, 0.1 M, pH 7.2	0.5 ml
Microsomes in 0.15 M KCl (equivalent to 200 mg tissue)	0.2 ml

A control flask was prepared containing all reactants except that 0.2 ml of 0.15 M KCl was added in place of liver microsomes. The open flasks were incubated with nitrogen as the gas phase in a Dubnoff shaking bath for 3 hours at 37 °C. At the end of the incubation period, the reaction was stopped by addition of 1.0 ml of 10% trichloroacetic acid to each flask. The flask contents were centrifuged for 20 minutes at 5000 RPM. The p-aminobenzoic acid formed in the reaction was measured by the procedures of Bratton and Marshall (19) and Fouts and Brodie (6), except that the following adjustments were made to accommodate the small volume

p-aminobenzoic acid standards were prepared and treated identically. After the incubation period, 2.5 ml iso-amyl alcohol was added and the color extracted. The tubes were centrifuged at 2000 rpm for 5-10 minutes to clear the phases. The alcoholic extract was read in semi-microcuvettes at 550 mμ in the Beckman DU Spectrophotometer.

Glucuronyl Transferase Activity of Liver Microsomes. Glucuronide synthesis was measured by the procedure of Levvy and Storey (20) as outlined in Bergmeyer (21). A microsomal suspension equivalent to 250 mg tissue was added to each reaction flask. A control flask contained all reactants except the microsomal suspension which was replaced by 0.15 M KCl. All were incubated in the Dubnoff shaking bath at 37°C for 30 minutes. The o-aminophenyl glucuronide formed from o-aminophenol as acceptor was estimated colorimetrically by the procedure outlined above for p-aminobenzoic acid, except that a 2-hour incubation in the dark was allowed before the extraction with iso-amyl alcohol.

Cytochrome P-450. The CO-binding pigment of liver microsomes was measured by the method of Dallner (22). 3 ml of a microsomal suspension was prepared in 0.05 M phosphate buffer, pH 7.5, so that 1 ml was equivalent to 100 mg tissue. Solid sodium dithionite was added to reduce

Alkaline Phosphatase Activity of Duodenal Tissue. Enzyme activity was estimated by the method of Lowry, et al (23), as described in Methods in Enzymology (24). The release of p-nitrophenol from the substrate, p-nitrophenyl-phosphate, was measured following incubation with an appropriately diluted aliquot of supernatant from the intestinal homogenate.

Trypsin Activity in Duodenal Contents. The supernatant obtained following centrifugation of pooled washes of the duodenal contents was assayed for tryptic activity by the method of Hummel (25). The rate of hydrolysis of the synthetic substrate, p-toluenesulfonyl-L-arginine methyl ester, was measured from the increase in absorbancy at 247 mμ. Total activity in units was calculated from the molar absorbancy of p-toluenesulfonyl-L-arginine and the protein content of the intestinal preparation.

Statistical Methods. Standard error of the mean was calculated for ungrouped data from control and treated animals on each experimental day. Level of significance was determined from the t-test for small samples.

Results and Discussion

Liver and Body Weight. Body weights of both control and experimental animals decreased slightly during the first twenty-eight hours (Table I, Figure 1), and liver weights decreased approximately thirty percent at the same time from a mean of 1.83 gm on zero day to 1.32 gm in twenty-four hours, and even lower in forty-eight hours (Table I, Figure 2). While the body weights of control animals then increased gradually to the eleventh day, as shown in Figure 1, the weights of animals drinking 30 ppm NTA remained relatively stable at the low level, so that by the seventh day the controls were significantly heavier than NTA-treated mice. By the fifteenth day, however, the NTA-treated mice had gained weight quite in excess of the control range. Subsequent measurements showed both groups to be alike.

Liver weights of control mice recovered rapidly within four days to zero day values, but those of mice drinking NTA remained depressed throughout the first week. By the seventh day of the experiment, the difference between the two groups was highly significant (Table I) with a mean of 2.09 gm for controls and 1.37 gm for treated mice. Measurements made from the eleventh day on showed no further differences between the two groups. Figure 3 shows the same pattern of change in the ratios of liver to body weight with significantly lower values in the NTA-treated animals on the fourth and seventh days (Table I).

The initial drop in body and liver weight may conceivably have resulted from dehydration if the animals found distilled water and the NTA solution distasteful at the outset of the experiment. There may also have been actual tissue loss if the animals failed to eat.

While total intake and output were not measured to determine water balance, the gross examination of Figure 4, showing a drastic depression in microsomal protein levels per liver during the first forty-eight hours, tends not to support the possibility of dehydration. The control animals fully returned to zero day levels forty-eight hours later. By the seventh day their liver microsomal protein levels were double those of the NTA-treated mice ($p = .005$). The microsomal protein levels of the treated animals did not approximate the control values until the eleventh day. This lag in recovery is quite strikingly apparent in Figure 4. Whether the unusually high values found for control animals on the seventh day resulted from chemical stimulation of the endoplasmic reticulum from an outside source is debatable, since the animals could not be fully protected from solvent fumes or other possible volatile stimulators, because of the type of ventilating system in the laboratory facility. If such stimulation occurred, it is interesting to observe that NTA appeared to inhibit the synthesis of microsomal protein at a time when control animals were achieving a remarkable recovery from the initial depression.

Cytochrome P-450 and Liver Microsomal Enzymes. The possibility that external chemical stimulation had occurred appears stronger upon examination of the Cytochrome P-450 results in Table II and Figure 5. Within twenty-four hours of the start of the experiment, a twenty to thirty percent increase in Cytochrome P-450 levels was observed in both the control and experimental groups. However, at no time during the entire experiment were statistically significant differences observed between the two groups in these particular determinations. Following

the initial rise, the values declined in both groups with a sustained lower, but not significant, level in the NTA-treated mice until the eleventh day, after which both groups were essentially the same.

Glucuronide formation, shown in Table II and Figure 6, followed no particular pattern. Activities in all animals decreased on the second day. On the fourth day the controls returned to zero day values. For the next three weeks, enzyme activity in the control mice was slightly lower, but quite constant. There was greater activity in the NTA-treated animals than controls on several days, with a greater mean and range of values on the fourth day. However, on this day the variance was too great for statistically significant differences to be demonstrated between the two groups. On the eleventh and twenty-ninth days, glucuronyl transferase activity in the treated animals was greater than in the controls ($p=<.05$).

Nitroreductase activity (Table II, Figure 7) generally fell into the pattern seen for microsomal protein levels with an initial decrease in all animals followed by a rapid increase in controls and a gradual rise in the NTA-treated mice. On the fourth day control levels were significantly elevated over those found in treated animals. From the eleventh day on, activity was greater in the treated mice, with mean values higher than those of controls on each sampling day. However, the longer the NTA treatment continued, the greater was the range of values for the group, so that no statistical significance could be attributed to the effects of NTA alone.

Had the liver microsomal system responded to nitrilotriacetic acid in the manner usually seen on administration of foreign compounds, the

cytochrome P-450 and . zyme activities in NTA-treated mice would have been expected to rise over control activities. Cytochrome P-450 usually increases rapidly within twenty-four to forty-eight hours in association with oxidative mechanisms related to drug metabolism, but in these experiments a rise was noted in both control and treated animals with no unusual elevation in the treated mice. Rise and decline in glucuronide formation and nitroreduction also were not demonstrated as being specifically related to NTA treatment. Although nitroreductase activity in treated mice showed a sustained, gradual increase to the fifteenth day from the initial decline, no increase over control activity occurred within the expected interval after the start of the experiment. Rather, the increase which was observed appeared related more to the change in liver size than to the NTA.

Intestinal Enzymes. Neither alkaline phosphatase nor trypsin activity (Table III) changed appreciably in the NTA-treated animals when compared to controls. Alkaline phosphatase (Figure 8) increased slightly in both groups at the outset and decreased after one week. Thereafter, the activity remained relatively constant in all animals. Trypsin in the duodenal washes (Figure 9) decreased during the first two days and then increased gradually to the fifteenth day, with the control levels ranging slightly higher than those of treated animals. These changes with time, especially in the treated mice, parallel those seen in Figure 4 (microsomal protein) and Figure 7 (nitroreductase) and further tend to support the possibility that they were related to food intake or assimilation rather than the experimental procedure.

SUMMARY AND CONCLUSIONS

In summary, the effects noted in control mice and mice drinking 30 ppm nitrilotriacetic acid are as follows:

1. The body and liver weights and total liver microsomal protein levels of all animals decreased within forty-eight hours of the start of the experiment. Controls recovered rapidly within the next two days. By the seventh day, body and liver weights of control mice were significantly greater than those of the NTA-treated animals, and liver microsomal protein levels in controls were found to be double those of mice drinking 30 ppm nitrilotriacetate.
2. Cytochrome P-450 increased 20 - 30% in all animals in twenty-four hours, suggesting a possible exposure to outside stimulation. No significant differences were observed between the two groups at any time.
3. Glucuronyl transferase activity appeared generally to be higher in NTA-treated animals, with a greater mean and range on the fourth day. However, only on the eleventh and twenty-ninth days was the difference significant.
4. Nitroreductase activity decreased in all animals within forty-eight hours, but it increased again rapidly in the controls and gradually in the experimental animals. Activity in control livers was significantly higher on the fourth day.
5. The activity of intestinal enzymes in treated mice did not vary appreciably from that of controls. Trypsin activity appeared to parallel the changes seen in liver microsomal

protein and nitroreductase, indicating that these differences may have been related to food intake rather than the experimental procedure.

While it appears probable that all animals in this experiment may have decreased their food intake in the first two days, the failure of NTA-treated mice to recover at the same rate as controls may have grave implications for the ability of exposed animals either to assimilate food or synthesize protein, or both. Not only did the liver and body weights remain low in mice drinking 30 ppm NTA, but their liver microsomal protein levels failed to increase as well during the first week of the experiment. Changes in enzyme activity were not typical of the responses usually observed upon stimulation of the liver microsomal system by foreign compounds.

In planning the original design for these experiments, it appeared that the effects of nitrilotriacetic acid on liver microsomal systems would best be determined by studying groups of animals subjected not to NTA alone, but also to a known stimulator such as phenobarbital and to phenobarbital in combination with NTA. Thus any possible inhibition or enhancement of the stimulatory process would become more apparent and provide more basic evidence of the type of response the liver makes to NTA. Given adequate personnel and facilities in which exposure of animals to outside stimulation is prevented, these experiments should be repeated with the additional controls as originally proposed.

Any further consideration of NTA as a potential additive to the environment should hinge on experiments designed to substantiate more fully whether NTA a) inhibits liver microsomal protein synthesis, b) inhibits the response of the microsomal system to known stimulators

such as phenobarbital, c) prevents weight gain in fasted animals on refeeding, d) adversely affects neonatal animals in synthesizing new protein and achieving growth.

A reduction in the capacity of the endoplasmic reticulum to synthesize enzymes could result in impairment of the detoxifying mechanisms and metabolic activity which permit an orderly utilization of drugs and enable the body to rid itself of unsuspected exposures to foreign compounds. On the other hand, enhancement of these processes could result in too rapid a turnover of normal body constituents, as well as an accelerated metabolism of drugs and organics, so multiplying the possibilities for increased accumulation of toxic end-products and increased occurrence of toxic interactions.

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TABLE 1

EFFECT OF NITRILOTRIACETIC ACID ON LIVER AND BODY WEIGHT

DAY	TREATMENT	n	LIVER WEIGHT GM	LIVER WEIGHT RANGE	BODY WEIGHT GM	BODY WEIGHT RANGE	LIVER/BODY WEIGHT %	LIVER/BODY WEIGHT RANGE
0	CONTROL	12	1.83±0.13	0.99-2.47	27.6±1.5	17.8-34.6	6.54±0.20	5.55-7.63
1	CONTROL	6	1.32±0.12	0.96-1.64	25.0±2.0	19.6-31.0	5.26±0.15	4.87-5.80
	NTA	5	1.32±0.12	0.95-1.70	26.7±1.5	22.8-29.8	4.97±0.47	3.19-5.71
2	CONTROL	6	1.17±0.06	1.02-1.36	23.6±1.1	21.1-28.6	4.95±0.10	4.72-5.45
	NTA	6	1.26±0.08	1.00-1.51	24.2±0.8	21.9-26.0	5.20±0.27	4.24-6.02
4	CONTROL	6	1.87±0.11	1.35-2.07	28.8±2.2	20.6-33.9	6.54±0.25	6.05-7.70
	NTA	6	1.31±0.11	0.99-1.60	25.1±1.5	20.0-29.4	5.24±0.15*	4.89-5.86
7	CONTROL	6	2.09±0.17	1.37-2.58	31.9±1.5	26.6-37.1	6.51±0.30	5.15-7.12
	NTA	6	1.37±0.07*	1.18-1.57	24.4±0.9*	22.2-28.0	5.65±0.23§	5.26-6.73
11	CONTROL	6	2.07±0.14	1.49-2.36	30.9±2.7	24.9-35.6	6.63±0.15	5.96-6.98
	NTA	6	1.81±0.17	1.33-2.37	28.3±1.5	24.5-34.0	6.30±0.27	5.43-7.06
15	CONTROL	6	1.82±0.14	1.25-2.15	29.2±1.0	25.8-31.9	6.20±0.41	4.85-7.57
	NTA	6	2.03±0.05	1.87-2.16	34.8±1.1†	30.8-37.7	5.86±0.17	5.33-6.46
29	CONTROL	6	1.50±0.14	1.16-2.06	27.5±2.4	20.7-37.6	5.47±0.11	5.13-5.84
	NTA	6	1.55±0.14	1.10-2.14	27.1±2.2	22.3-35.8	5.58±0.21	4.78-6.28
45	CONTROL	6	1.78±0.15	1.38-2.25	31.2±1.9	26.1-36.7	5.59±0.25	4.85-6.44
	NTA	6	1.63±0.04	1.53-1.79	30.3±1.3	26.8-34.0	5.43±0.23	4.61-6.12

* p=<.005 Value is the mean ± standard error.
† p=<.001
§ p=<.05

EFFECT OF NITRILOTRIACETIC ACID ON LIVER MICROSOMAL ENZYMES
AND CYTOCHROME P-450

DAY	TREATMENT	n	CYTOCHROME P-450 ΔOD(450mu)[1]	RANGE	GLUCURONYL TRANSFERASE GLUCURONIDE FORMED[2]	RANGE	NITROREDUCTASE PABA FORMED[3]	RANGE
0	CONTROL	12	1.03±0.08	0.44-1.38	1.211±0.124	0.913-1.728	0.270±0.060	0.074-0.860
1	CONTROL	6	1.32±0.12	0.83-1.62	1.179±0.212	0.448-1.712	0.123±0.021	0.054-0.190
	NTA	5	1.24±0.07	1.10-1.48	1.284±0.170	0.992-1.600	0.116±0.010	0.084-0.142
2	CONTROL	6	1.10±0.14	0.58-1.55	0.856±0.082	0.512-1.056	0.104±0.007	0.074-0.120
	NTA	6	0.90±0.10	0.58-1.28	0.787±0.225	0.288-1.792	0.137±0.031	0.050-0.260
4	CONTROL	6	1.12±0.16	0.65-1.56	1.133±0,082	0.800-1.360	0.352±0.024	0.264-0.432
	NTA	6	0.95±0.14	0.60-1.50	1.453±0.180	0,960-2.240	0.183±0.034*	0.088-0.314
7	CONTROL	6	1.16±0.16	0.62-1.58	0.976±0.079	0.736-1.232	0.327±0.038	0.188-0.452
	NTA	6	0.89±0.06	0.72-1.08	0.944±0.111	0.592-1.424	0.284±0.052	0.120-0.360
11	CONTROL	6	1.13±0.09	0.85-1.43	0.963±0,085	0.768-1.344	0.211±0.009	0.186-0.248
	NTA	6	1.26±0.05	1.08-1.45	1.285±0.116§	0,992-1.824	0.367±0,103	0.146-0.840
15	CONTROL	6	1.23±0.16	0.60-1.80	0.947±0.056	0.800-1.136	0.412±0.094	0.192-0.836
	NTA	6	1.19±0.10	0.85-1.43	1.083±0.140	0.720-1.616	0.449±0.079	0.296-0.776
29	CONTROL	6	0.74±0.08	0.51-0.98	0.891±0.052	0.688-1.024	0.121±0.022	0.078-0.214
	NTA	6	0.89±0.07	0.66-1.06	1.165±0.110§	0.848-1.632	0.373±0.140	0.048-1.006
45	CONTROL	6	1.16±0.09	0.90-1.48	1.805±0.224	1.168-2.736	0.339±0.058	0.158-0.526
	NTA	6	1.06±0.07	0.74-1.20	1.744±0.233	1.232-2.720	0.475±0.146	0.188-1.076

1 From difference-spectra (reduced-reduced (CO)) of microsomal fraction of 1 g. liver.
2 Expressed as OD units per 1 g. liver.
3 Expressed as micromoles of ρ-aminobenzoic acid formed per 1 g. liver.
* p = <.005 Value is the mean ± standard error.
§ p = <.05

TABLE III

EFFECT OF NITRILOTRIACETIC ACID ON ACTIVITY OF INTESTINAL ENZYMES

DAY	TREATMENT	n	ALKALINE PHOSPHATASE MILLIMOLAR UNITS[1]	ALKALINE PHOSPHATASE RANGE	TRYPSIN MICROMOLAR UNITS[2]	TRYPSIN RANGE
0	CONTROL	12	6.13±0.46	3.73- 8.69	6.03±0.83	1.45- 9.48
1	CONTROL	6	7.28±0.92	4.62-11.32	5.45±1.00	3.45- 9.81
	NTA	5	6.52±1.25	3.22-10.38	3.70±0.54	1.99- 5.11
2	CONTROL	6	7.11±0.46	5.56- 8.33	3.33±0.46	2.59- 5.40
	NTA	6	7.49±0.26	6.44- 8.45	4.38±0.51	3.52- 6.84
4	CONTROL	6	7.69±0.84	4.25- 9.68	5.19±0.60	3.63- 6.59
	NTA	6	5.93±0.64	3.10- 7.58	4.33±1.27	3.04-10.20
7	CONTROL	6	5.97±0.47	4.75- 8.15	6.04±1.31	2.42-11.82
	NTA	6	5.28±0.33	4.16- 6.29	5.45±0.69	3.20- 8.21
11	CONTROL	6	6.56±1.18	6.14-10.42	8.41±1.34	4.98-11.63
	NTA	6	6.61±0.23	5.68- 8.43	6.86±0.75	4.88- 9.50
15	CONTROL	6	6.29±0.52	4.34- 7.61	7.54±1.03	4.33-10.53
	NTA	6	7.22±0.52	5.91- 9.25	9.03±0.94	6.71-12.73
29	CONTROL	6	5.96±0.39	5.08- 6.93	6.94±0.65	4.91- 9.20
	NTA	6	6.59±0.84	4.21- 9.04	6.81±0.93	4.02- 9.93
45	CONTROL	5	7.53±0.78	5.48- 9.98	10.85±1.18	8.20-15.13
	NTA	6	6.48±1.21	6.36-10.22	8.54±0.43	7.33- 9.96

[1] Enzyme activity is expressed as millimoles ρ-nitrophenyl phosphate hydrolyzed per 1 g. intestine per hour.

[2] Enzyme activity is expressed as micromoles of ρ-toluene-sulfonyl-L-arginine methyl ester hydrolyzed per mg. protein per minute. Total activity is calculated from the protein content of washes of an 8 cm segment of duodenum.

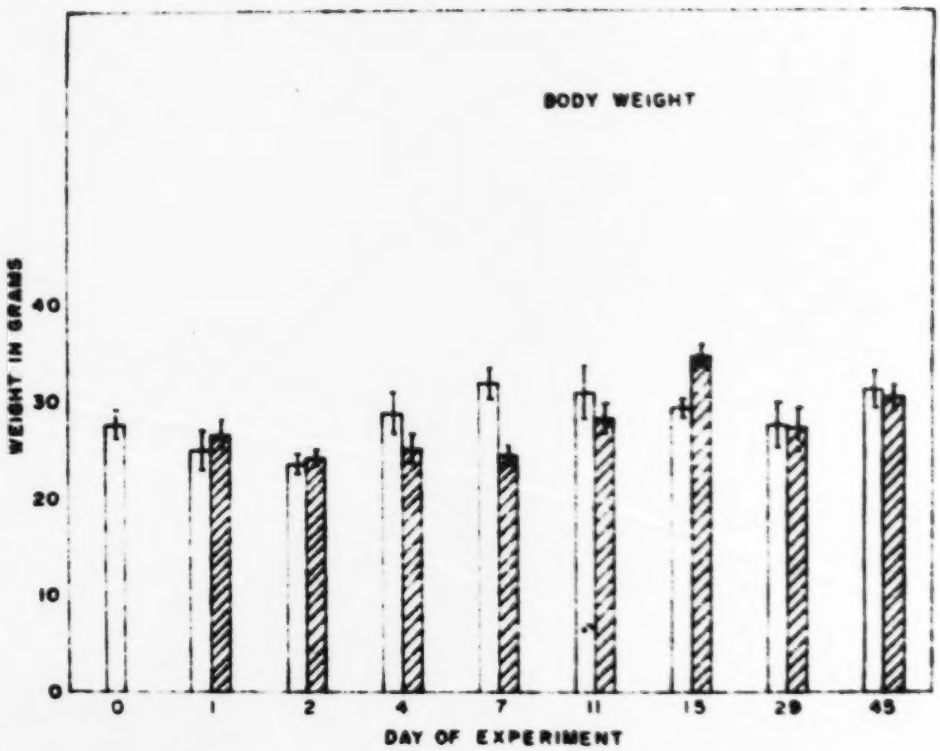

Figure 1. Body weights of control mice (open bars) and mice drinking 30 ppm nitrilotriacetate (cross-hatched bars) during the forty-five day experiment. Differences were significant on the seventh and fifteenth days.

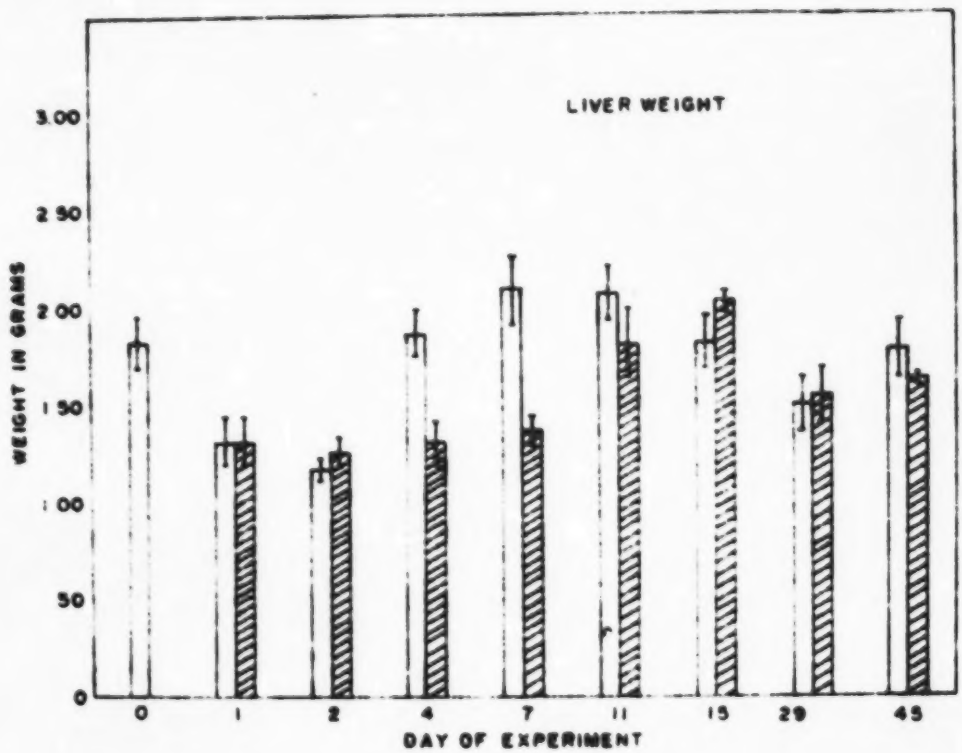

Figure 2. Liver weights of control (open bars) and experimental (cross-hatched bars) mice. Livers of NTA-treated mice were smaller on the fourth and seventh ($p < .005$) days.

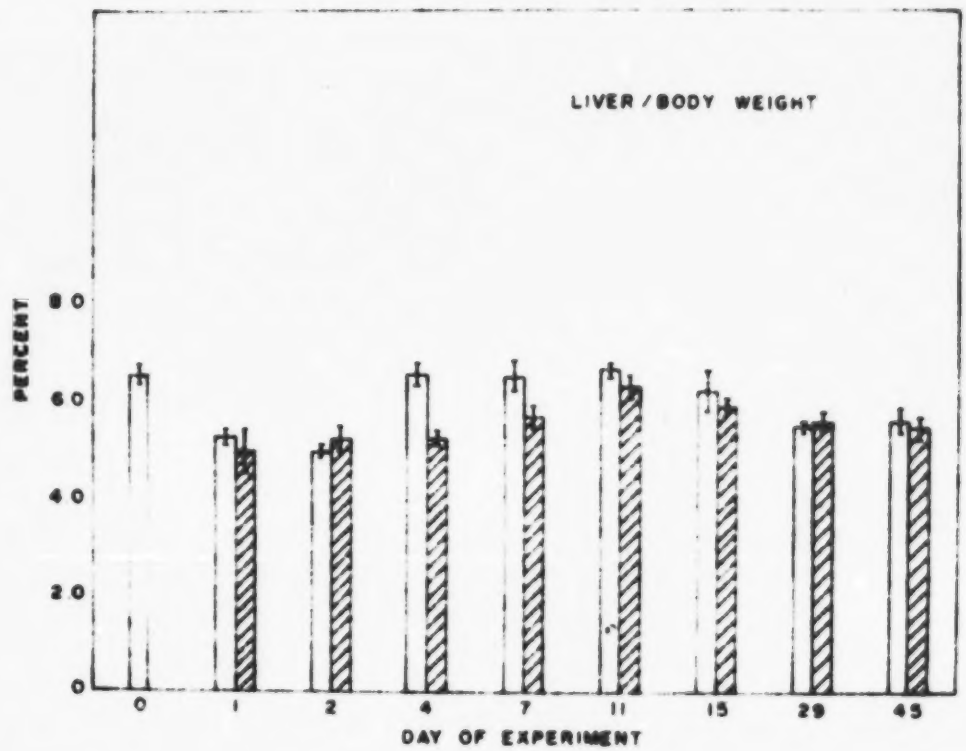

Figure 3. Ratios of liver to body weights were significantly lower in NTA-treated mice (cross-hatched bars) on the fourth and seventh days.

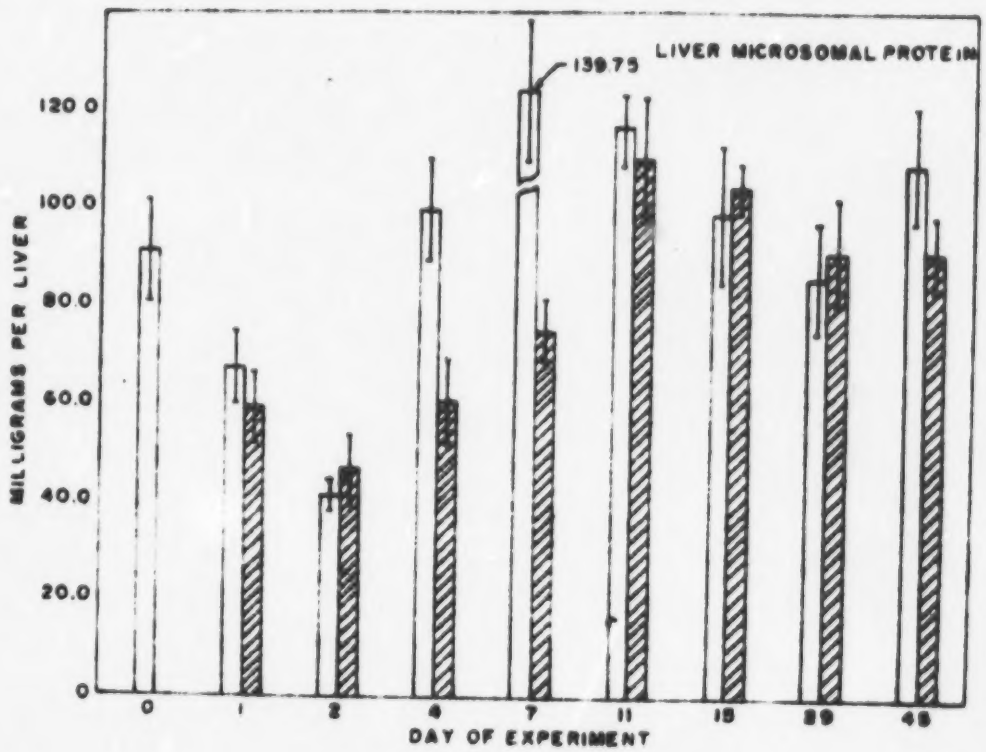

Figure 4. Microsomal protein levels calculated per liver in control (open bars) and experimental mice, with control values nearly double those of NTA-treated animals ($p < .005$).

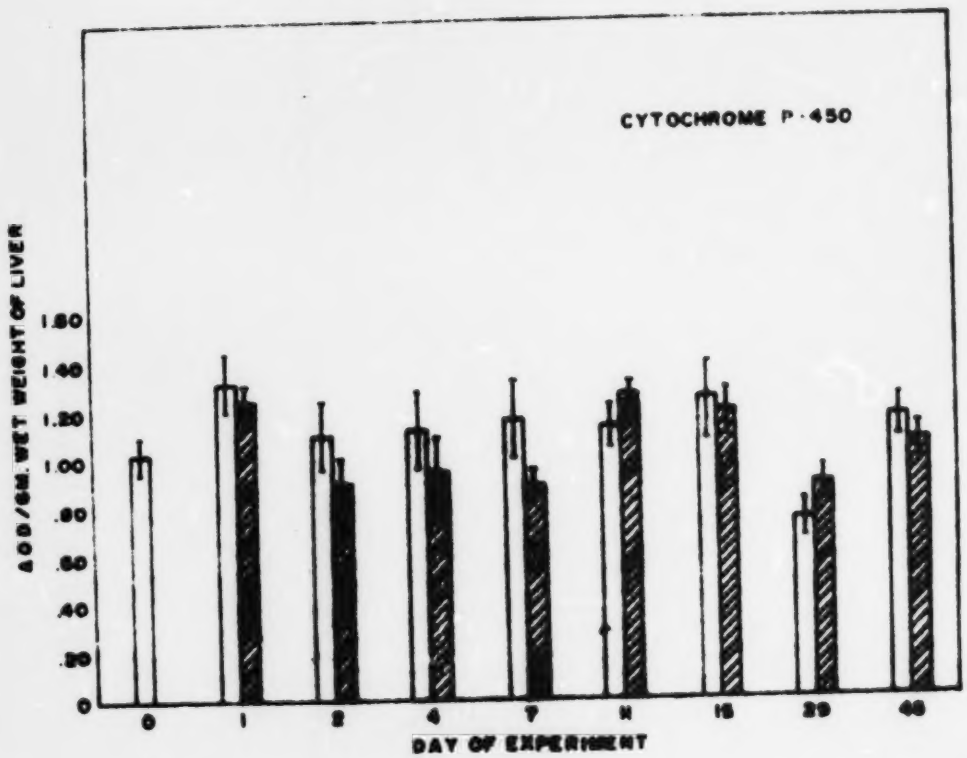

Figure 5. Liver microsomal cytochrome P-450. Differences between control (open bars) and treated (cross-hatched bars) are not significant.

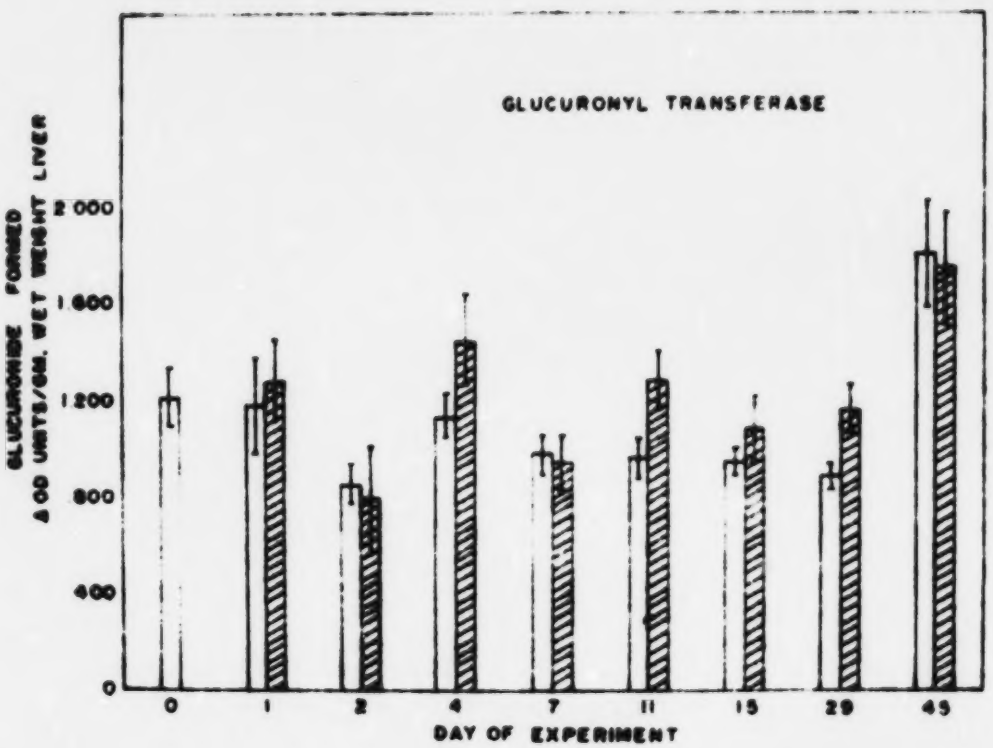

Figure 6. Glucuronide synthesis by liver microsomes from o-aminophenol as substrate. Differences in NTA-treated mice (cross-hatched bars) are significant at the $\alpha = .05$ level on the eleventh and twenty-ninth days.

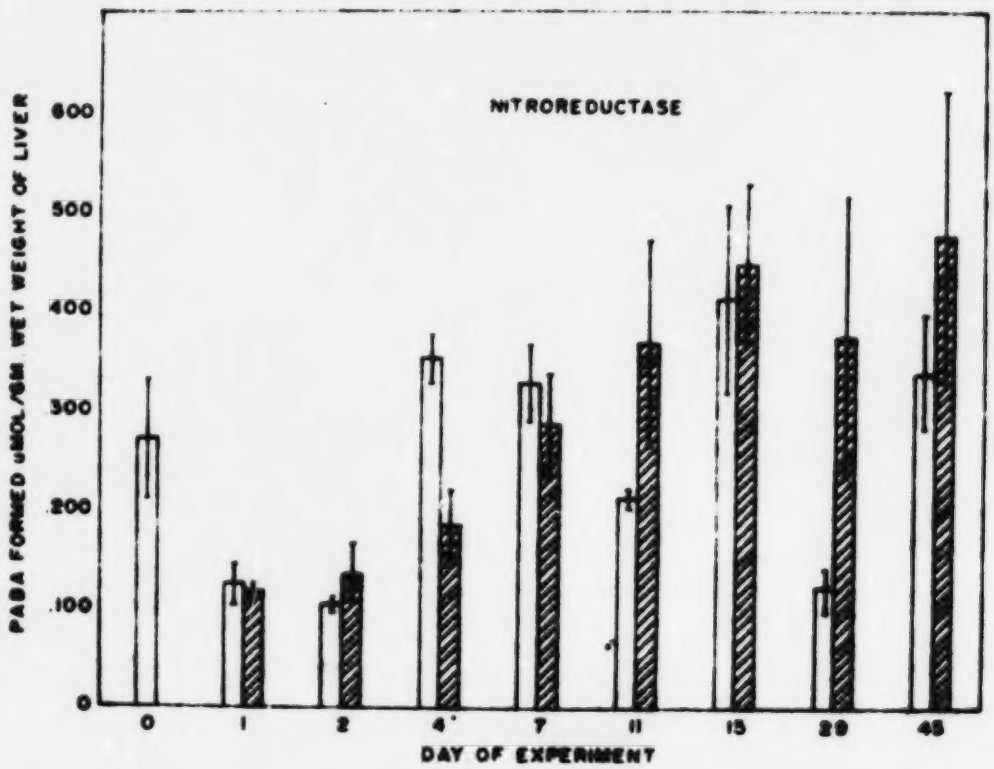

Figure 7. Nitroreductase activity of liver microsomes. p-Aminobenzoic acid formed by control livers (open-bars) was significantly higher on the fourth day.

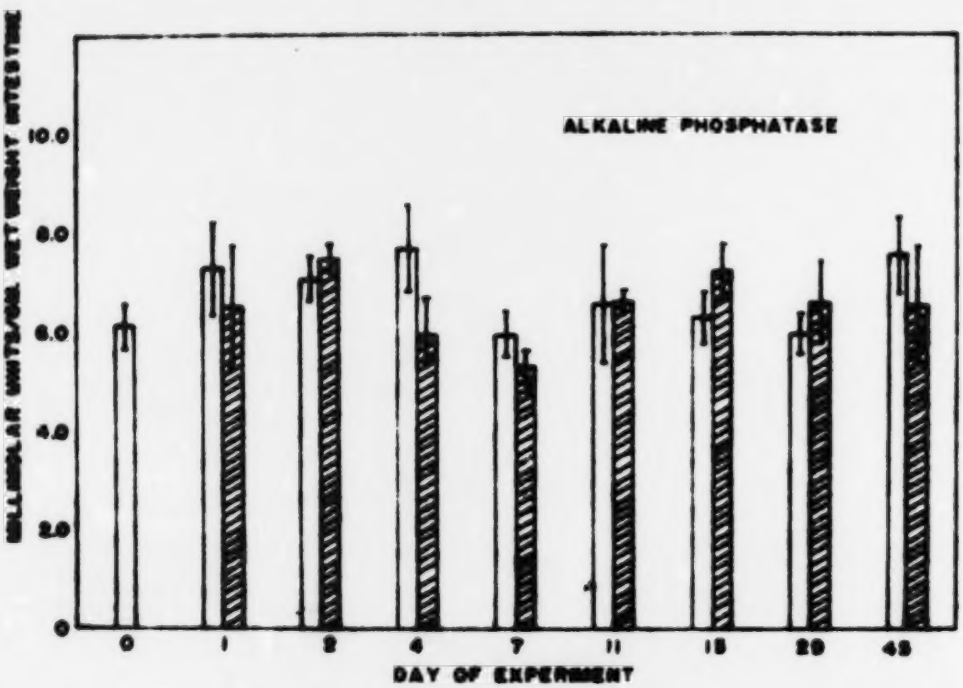

Figure 9. Alkaline phosphatase activity in duodenum of control (open-bars) and NTA-treated (cross-hatched bars) mice.

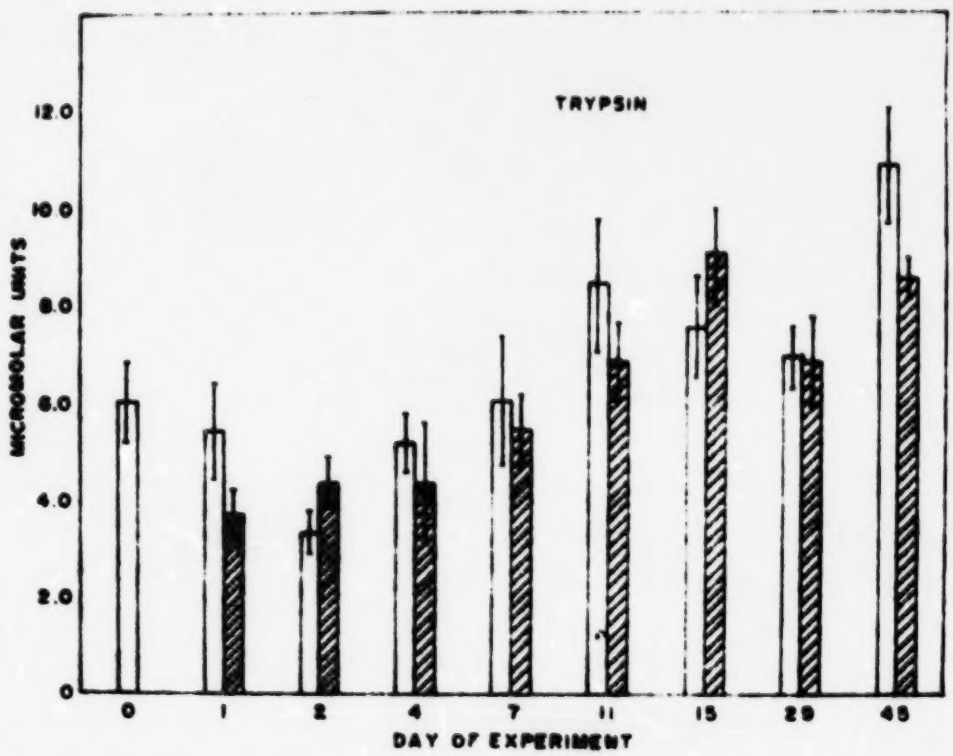

Figure 9. Trypsin activity in duodenal washes of control (open bars) and NTA-treated (cross-hatched bars) mice.